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Invention: MULTI-MER PEPTIDES DERIVED FROM HEPATITIS C VIRUS ENVELOPE PROTEINS FOR DIAGNOSTIC USE AND VACCINATION PURPOSES

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SPECIFICATION

MULTI-MER PEPTIDES DERIVED FROM HEPATITIS C VIRUS ENVELOPE PROTEINS FOR DIAGNOSTIC USE AND VACCINATION PURPOSES

FIELD OF THE INVENTION

The present invention relates to multi-mer peptides derived from hepatitis C virus envelope proteins which react with the majority of anti-HCV antibodies present in patient sera. Consequently, the present invention relates to the usage of the latter peptides to diagnose, and to vaccinate against, an infection with hepatitis C virus.

BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) infection is a major health problem in both developed and developing countries. It is estimated that about 1 to 5 % of the world population is affected by the virus, amounting up to 175 million chronic infections worldwide. HCV infection appears to be the most important cause of transfusion-associated hepatitis and frequently progresses to chronic liver damage. Moreover, there is evidence implicating HCV in induction of hepatocellular carcinoma. Consequently, the demand for reliable diagnostic methods and effective therapeutic agents is high. There is also an urgent need to characterize new epitopes which can be used in the design of effective vaccines against hepatitis C.

HCV is a positive stranded RNA virus of about 9,8 kilobases which code for at least three structural and at least six non-structural proteins. The structural proteins have not yet been functionally assigned, but are thought to consist of a single core protein and two envelope proteins E1 and E2. The E1 protein consists of 192 amino acids and contains 5 to 6 N-glycosylation sites, depending on the HCV genotype, whereas the E2 protein consists of 363 to 370 amino acids and contains up to 11 N-glycosylation sites, depending on the HCV genotype (for review see Maertens and Stuyver, 1997).

The E1 and E2 proteins are currently not included in HCV antibody (Ab) assays, primarily because of their complex conformational structures which require expression in mammalian cells as well as non-denaturing purification techniques. Indeed, after expression of E2 in *Escherichia coli*, the reactivity of HCV sera with the recombinant protein ranged from 14

(Yokosuka et al., 1992) to 17 % (Mita et al., 1992), whereas expression in eukaryotic systems yields reactivities of 13 to 97 % (Inoue, 1992; Chien, 1993). Others demonstrated that the E1 protein expressed as a single protein from eukaryotic cells did not shown high reactivity with patient sera (from 6 to 60%; Kohara et al. (1992), Hsu et al. (1992), Chien et al. (1993)). We previously reported that high prevalences of Ab's to both of the purified recombinant E1 and E2 proteins, which were expressed in mammalian cells, could be found in sera from chronic hepatitis C patients (WO 96/04385 to Maertens et al.). In this regard, we also demonstrated that the majority of anti-E1 and anti-E2 antibodies in sera from HCV patients could not be mapped using 20-mer peptides (WO 96/04385 to Maertens et al.). Indeed, although all of the murine monoclonal Ab's against E1 could be mapped to reactivity with two 20-mer peptides, denoted as epitope A (amino acids (aa) 313-326) and epitope B (aa 208-224), at most 50 % of patient sera reactive with recombinant proteins recognized epitope A and B. With regard to the E2 protein, only three out of twenty four murine monoclonal Ab's could be mapped using 20-mer peptides. These three Ab's were mapped to the hypervariable region I (HVR I) covered by peptide E2-67 (aa 394-413) and to a region covered by a peptide denoted E2-13B (aa 523-542). The remaining twenty-one Ab's could not be mapped using 20-mer peptides. The relative map positions of seven of these Ab's could be deduced from competition studies using recombinant E2 protein.

Taken together, it appears that anti-E1 and anti-E2 Ab's might be highly prevalent in sera of HCV patients. However, determining the presence of these Ab's is problematic due to the need to use eukaryotically expressed E1 and E2, which have to be purified using cumbersome non-denaturing techniques. As an alternative, chemically synthesized 20-mer peptides derived from the E1 and/or E2 proteins were produced. However, these synthesized 20-mer peptides were not able to recognize the anti-E1 and anti-E2 Ab's in sera from HCV patients.

There is thus a need to design alternative methods to screen for HCV envelope Ab's.

AIMS OF THE INVENTION

It is clear from the literature cited above that the E1 and E2 proteins probably have complex conformational structures which are essential for recognizing (and binding to) the anti-E1 and anti-E2 Ab's in sera from HCV patients. This could explain why prokaryotically

expressed complete or near-complete E1 and E2 proteins, which might be malfolded due to the lack of glycosylations, relevant chaperones or correct cysteine bridges, and 20-mer peptides, which might be unable to mimic a complex conformational structure, are not able to recognize these Ab's.

The present invention relates to the surprising finding that multi-mer peptides (eg 30- to 45-mer peptides) are able to recognize the majority of anti-E1 and anti-E2 Ab's in sera from HCV patients. It should be clear that this is a surprising finding because there is no guidance which would suggest that 30- to 45-mer peptides derived from E1 and E2 would acquire proper folding and would efficiently recognize the majority of HCV envelope Ab's. In contrast, one would assume that the chance that multi-mer peptides malfold would be as great, or even greater, than the chance that prokaryotically expressed complete proteins malfold as is suggested above. In the case of the HCV NS3 protein for example, which reacts with more than 90 % of patient samples as expressed from *E. coli,* 20-50 mer peptides only react very weakly.

Therefore, the present invention aims at providing a peptide of more than 20 contiguous amino acids derived from the envelope region of HCV-related viruses which binds and recognizes anti-HCV-related virus antibodies. HCV-related viruses, including HCV, GBV-B virus, GBV-A virus and GBV-C (HGV or hepatitis G virus), are a division of the Flaviviruses, which further comprise Dengue virus, Yellow fever virus, Pestiviruses such as Classical Swine Fever Virus and Bovine Viral Diarrhea Virus (Wengler, 1991).

More specifically, the present invention aims at providing a peptide which binds and recognizes an anti-HCV antibody or an anti-HGV antibody present in a sample of body fluid and which is chosen from the group consisting of the sequences as represented in SEQ ID NOs 1 to 38 (see Table 1) or a functionally equivalent variant or fragment thereof.

In this respect, the present invention aims specifically at providing a peptide as described above, wherein said anti-HCV antibody present in a sample of body fluid is an anti-HCV-E1antibody or an anti-HCV-E2 antibody.

The present invention thus aims also at providing a peptide as described above, wherein said anti-

HGV antibody present in a sample of body fluid is an anti-HGV-E1 antibody or an anti-HGV-E2 antibody.

Moreover, the present invention aims at providing a peptide as described above, wherein said peptide is synthesized chemically or is synthesized using recombinant DNA techniques.

The present invention also aims at providing a peptide as described above, wherein said peptide is biotinylated or contains cysteine bridges.

Furthermore, the present invention aims at providing any combination of peptides as described above, as well as compositions containing said combination of peptides or peptides as described above .

In addition, the present invention aims at providing a method for diagnosing exposure to or infection by HCV-related viruses comprising contacting anti-HCV-related virus antibodies within a sample of body fluid with a peptide as described above or with a combination of peptides as described above, determining the binding of anti-HCV-related virus antibodies within a sample of body fluid with a peptide as described above or with a combination of peptides as described above.

In addition, the present invention aims at providing an assay kit for detecting the presence of anti-HCV-related virus antibodies within a sample of body fluid comprising a solid support, a peptide as described above or a combination of peptides as described above, appropriate markers which allow to determine the complexes formed between anti-HCV-related virus antibodies within a sample of body fluid with a peptide as described above or a combination of peptides as described above.

In addition, the present invention aims at providing a bioassay for identifying compounds which modulate the interaction between a peptide and an anti-HCV-related virus antibody, said bioassay comprising contacting anti-HCV-related virus antibodies with a peptide as described above or a combination of peptides as described above, determining the binding of anti-HCV-related virus

antibodies with a peptide as described above or a combination of peptides as described above, adding a modulator (ie a compound which is able to modulate the interaction between an envelope protein and an anti-HCV-related virus antibody) or a combination of modulators to the contacted anti-HCV-related virus antibodies with a peptide as described above or a combination of peptides as described above, determining the modulation of binding of anti-HCV-related virus antibodies with a peptide as described above or a combination of peptides as described above

In addition, the present invention aims at providing a bioassay for identifying compounds which modulate the interaction between a peptide and an anti-HCV-related virus antibody, said bioassay comprising determining the binding of anti-HCV-related virus antibodies with a peptide as described above or a combination of peptides as described above, contacting a modulator with a peptide as described above or a combination of peptides as described above, adding anti-HCV-related virus antibodies to the contacted modulator with the peptide as described above or a combination of peptides as described above, determining the modulation of binding between anti-HCV-related virus antibodies with a peptide as described above or a combination of peptides as described above.

Moreover, the present invention aims at providing a modulator, a composition containing a modulator, or a combination of modulators when produced by the bioassay as described above or when identified by the above-described bioassays.

Moreover, the present invention aims at providing a composition comprising a plasmid vector comprising a nucleotide sequence encoding a peptide as described above, or a modulator as described above, operably linked to transcription regulatory elements.

Moreover, the present invention aims at providing a composition as described above for use to vaccinate or therapeutically treat humans against infection with HCV-related virus or any mutated strain thereof.

Moreover, it is an aim of the present invention to provide an antibody, more particularly a monoclonal antibody, characterized in that it specifically recognizes an HCV-related virus

polypeptide as described above.

Finally, it is an aim of the present invention to provide a method to immunize humans against infection with HCV-related virus or any mutated strain thereof, comprising the use of a peptide as described above or a combination of peptides as described above.

All the aims of the present invention are considered to have been met by the embodiments as set out below. Other advantages and features of the instant invention will be evident from the following claims and detailed description.

BRIEF DESCRIPTION OF TABLES AND DRAWINGS

Table 1 provides information on the envelope protein and the HCV genotype from which the peptides of the present invention are derived. This table also provides the name, the amino acid sequence, the position within the envelope proteins and the sequence identity (SEQ ID) of the peptides of the present invention.

Table 2 shows ELISA results (in mOD) of reactivities of multimer peptides and recombinant E2 with 60 HCV positive samples and 4 control samples.

Table 3 shows the analysis for E1 antibodies of 23 sera from responders to interferon treatment.

Table 4 shows the analysis of E2 antibodies of 23 sera from responders to interferon treatment.

Table 5 shows the monitoring of disease over time by measuring antibodies to the HCV E1 and E2 regions in 18 patients.

Table 6 indicates the reactivity of HGV (Hepatitis G virus) RNA positive sera with the HGV E1 peptide V1V2.

Figure 1 demonstrates the positions of the multi-mer peptides of the present invention relative to the conserved and variable regions of the E1 envelope protein of HCV (HVR = hypervariable regions; V = variable regions; C = conserved regions; HR= hydrophobic region; SA = signal anchor domain; Y = glycosylation; **I** = cysteine).

Figure 2 demonstrates the positions of the multi-mer peptides of the present invention relative to the conserved and variable regions of the E2 envelope protein of HCV (HVR = hypervariable regions; V = variable regions; C = conserved regions; SA = signal anchor domain; Y = glycosylation; **I** = cysteine).

Figure 3 shows the reactivity of 20-mer E2 peptides. The OD values of serum samples from patients with chronic active hepatitis C were added and plotted against the different peptides.

Figure 4 shows the reactivity of mulit-mer E2 peptides. The OD values of the samples were added and plotted against the different peptides. The samples were identical as used for Figure 3.

DETAILED DESCRIPTION OF THE INVENTION

The invention described herein draws on previously published work and pending patent applications. By way of example, such work consists of scientific papers, patents or pending patent applications. All these publications and applications, cited previously or below are hereby incorporated by reference.

The present invention is based on the finding that multimer peptides, as of a certain length, derived from the envelope proteins of HCV-related viruses, eg HCV and HGV, recognize and bind anti-HCV-related virus antibodies, eg anti-HCV antibodies and anti-HGV antibodies, respectively. Therefore, the present invention provides a peptide of more than 20 contiguous amino acids derived from the envelope region of HCV-related viruses which binds and recognizes anti-HCV-related virus antibodies.

HCV-related viruses include, but are not limited to HCV, GBV-B virus, GBV-A virus and GBV-C virus (HGV or hepatitis G virus)(Linnen et al., 1996). HCV constitutes a genus within the Flaviviridae, and is closely related to hepatitis G virus (26.8 % at the amino acid level).
The term "envelope region" of HCV-related viruses is a well-known region by a person skilled in the art (Wengler, 1991), and comprises the E1 protein as well as the E2 protein, which was previously called non-structural protein 1 (NS1) or E2/NS1.

Furthermore, the present invention relates to a peptide, which binds and recognizes an anti-HCV antibody or an anti-HGV antibody present in a sample of body fluid, and which is chosen from the group consisting of the sequences as represented in SEQ ID 1 to 38 (see Table 1) or a functionally equivalent variant or fragment thereof.

The present invention relates also to a peptide as described above, wherein said anti-HCV antibody or said anti-HGV antibody present in a sample of body fluid is an anti-HCV-E1 or anti-HCV-E2 antibody, or an anti-HGV-E1 or anti-HGV-E2 antibody, respectively.

The term "a peptide" refers to a polymer of amino acids (aa's) derived (i.e. containing less aa's)

from the well-known HCV-related virus envelope proteins E1 and E2 (Linnen et al., 1996, Maertens and Stuyver, 1997), which binds anti-HCV-related virus antibodies. The term "a peptide" refers in particular to a polymer of aa's derived from HCV envelope proteins E1 and E2, which binds anti-HCV antibodies, or from HGV envelope proteins E1 and E2, which binds anti-HGV antibodies.

The terms "peptide", "polypeptide" and "protein" are used interchangeably herein.

The term "an anti-HCV-related virus antibody" refers to any polyclonal or monoclonal antibody binding to a HCV-related virus particle or any molecule derived from said viral particle. More particularly, the term "an anti-HCV-related virus antibody" refers to antibodies binding to the natural, recombinant or synthetic E1 and/or E2 proteins derived from HCV or HGV proteins (anti-HCV-E1 or anti-HCV-E2 antibody, or anti-HGV-E1 or anti-HGV-E2 antibody, respectively).

The term "monoclonal antibody" used herein refers to an antibody composition having a homogeneous antibody population. The term is not limiting regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made.

In addition, the term "antibody" also refers to humanized antibodies in which at least a portion of the framework regions of an immunoglobulin are derived from human immunoglobulin sequences and single chain antibodies as described in U.S. patent N° 4,946,778 and to fragments of antibodies such as F_{ab}, $F_{(ab)2}$, F_v and other fragments which retain the antigen binding function and specificity of the parent antibody.

The term " a sample of body fluid" as used herein refers to a fluid obtained from an organism, such as serum, plasma, saliva, gastric secretions, mucus, spinal cord fluid, and the like.

The term "the group consisting of the sequences as represented in SEQ ID NOs 1 to 38" as used herein refers to the thirty-eight peptides shown in Table 1 of the present application. In this table, it is indicated:

- in the column named "protein" from which HCV envelope protein the peptide is derived, but for the envelope protein of HGV, which is denoted E1(HGV),
- in the column named "genotype" the HCV genotype from which the envelope protein is derived, and thus the peptide is derived, except for HGV which was not determined (ND),
- in the column named "peptide" the assignment of the peptide region.

- the aa sequence of the peptide and,
- in the column named "position", the well-known (Maertens and Stuyver, 1997) aa position of the peptides within the HCV envelope proteins. Note that the position for the E1 envelope protein is not determined, which is denoted as "ND".

The term "functionally equivalent" as used in "functionally equivalent variant or fragment thereof" refers to variants and fragments of the peptides represented by SEQ ID 1 to 38, which bind anti-HCV-related virus antibodies. The term "variant or fragment" as used in "functionally equivalent variant or fragment thereof" refers to any variant or any fragment of the peptides represented by SEQ ID 1 to 38. Furthermore, the latter terms do not refer to, nor do they exclude, post-translational modifications of the peptides represented by SEQ ID 1 to 38 such as glycosylation, acetylation, phosphorylation, modifications with fatty acids and the like. Included within the definition are, for example, peptides containing one or more analogues of an aa (including unnatural aa's), peptides with substituted linkages, mutated versions or natural sequence variations of the peptides (for example ~~corres~~ponding to the genotypes HCV, as described in WO 94/12670 to Maertens et al.), peptides containing disulfide bounds between cysteine residues, or other cysteine modifications, biotinylated peptides, as well as other modifications known in the art. Modification of the structure of the polypeptides can be for such objectives as increasing therapeutic or prophylactic efficacy, stability (e.g. ex vivo shelf life and in vivo resistance to proteolytic degradation), or post-translational modifications (e.g. to alter the phosphorylation pattern of protein). Such modified peptides, when designed to retain at least one activity of the naturally-occurring form of the protein are considered functional equivalents of the polypeptides described in more detail herein. Such modified peptides can be produced, for instance, by amino acid substitution, deletion, or addition. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (i.e. isosteric and/or isoelectric mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids can be divided into four families: (1) acidic: aspartate, glutamate; (2) basic: lysine, arginine, histidine; (3) nonpolar: alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar: glycine, asparagine, glutamine, cysteine, serine, threonine,

tyrosine. In similar fashion, the amino acid repertoire can be grouped as (1) acidic: aspartate, glutamate; (2) basic: lysin, arginine histidine, (3) aliphatic: glycine, alanine, valine, leucine, isoleucine, serine, threonine, with serine and threonine optionally be grouped separately as aliphatic-hydroxyl; (4) aromatic: phenylalanine, tyrosine, tryptophan; (5) amide: asparagine, glutamine; and (6) sulfur-containing: cysteine and methionine. (see, for example, Biochemistry, 2nd ed., Ed. by L. Stryer, WH Freeman and Co.: 1981). Whether a change in the amino acid sequence of a peptide results in a functional homologue (e.g. functional in the sense that the resulting polypeptide mimics the wild-type form) can be readily determined by assessing the ability of the variant peptide to produce a response in e.g. ELISAs in a fashion similar to the wild-type protein, or to competitively inhibit such a response. Polypeptides in which more than one replacement has been introduced can be readily tested in the same manner.

It should also be clear that the region of a peptide represented by SEQ ID 1 to 38 which bind to an antibody (the so-called epitope) need not to be composed of a contiguous aa sequence.

In this regard, the term "fragment" includes any fragment which comprises these non-contiguous binding regions or parts thereof. In other words, fragments which include these binding regions may be separated by a linker, which is not a functional part of the epitope. This linker does not need to be an amino acid sequence, but can be any molecule, eg organic or inorganic, that allows the formation of the desired epitope by two or more fragments.

Moreover, it should be clear that the variants and fragments of SEQ ID NOs 1 to 5, 7 to 9, and 18 as used herein include peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's, or 34 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 6 as used herein include to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 10 as used herein include to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NOs 11, 15, 21, 34 as used herein include to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NOs 12, 24 or 32 as used herein include to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's,

or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's, or 34 aa's, or 35 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NOs 13, 22, or 34 used herein include to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's. Moreover, it should be that clear the variants and fragments of SEQ ID NO 16 as used herein include to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's, or 34 aa's, 35 aa's, or 36 aa's, or 37 aa's, or 38 aa's, or 39 aa's, or 40 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 17 as used herein refers to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 19 as used herein refers to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's, or 34 aa's, 35 aa's, or 36 aa's, or 37 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NOs 20 and 30 as used herein include to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 23 as used herein include to peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's, or 34 aa's, 35 aa's, or 36 aa's, or 37 aa's, or 38 aa's, or 39 aa's, or 40 aa's, or 41 aa's, or 42 aa's, or 43 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NOs 25 or 29 as used herein include peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 26 as used herein include peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 27 as used herein include peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's, or 34 aa's, 35 aa's, or 36 aa's, or 37 aa's, or 38 aa's, or 39 aa's, or 40 aa's, or 41 aa's, or 42 aa's, or 43 aa's, or 44 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 28 or 31 as used herein include peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32

aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NO 33 as used herein include peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's, or 34 aa's, 35 aa's, or 36 aa's, or 37 aa's, or 38 aa's, or 39 aa's, or 40 aa's, or 41 aa's. Moreover, it should be clear that the variants and fragments of SEQ ID NOs 14 or 37 as used herein include peptides having a length of at least 21 aa's, or 22 aa's, or 23 aa's, or 24 aa's, or 25 aa's, or 26 aa's, or 27 aa's, or 28 aa's, or 29 aa's, or 30 aa's, or 31 aa's, or 32 aa's, or 33 aa's, or 34 aa's, 35 aa's, or 36 aa's, or 37 aa's, or 38 aa's, or 39 aa's.

In addition, it shall be appreciated by the person skilled in the art that the amino acid regions of the peptides, which are disclosed in the present invention and that bind anti-HCV antibodies, can be delineated in more detail by experimentation.

In addition, it should be clear that the variants and fragments of the peptides represented by SEQ ID 1 to 38, as herein described, can be prepared by any method known in the art such as classical chemical synthesis, as described by Houbenweyl (1974) and Atherton & Shepard (1989), or by means of recombinant DNA techniques as described by eg Maniatis et al. (1982), or Sambrook et al. (1989).

Similarly, it should be clear that also the peptides represented by SEQ ID 1 to 38 of the present invention can be prepared by any method known in the art and more particularly by means of classical chemical synthesis, as described by Houbenweyl (1974) and Atherton & Shepard (1989), or by means of recombinant DNA techniques such as described by eg Maniatis et al. (1982), or Sambrook et al. (1989).

The present invention further relates to the peptides represented by SEQ ID 1 to 38 and functionally equivalent variants or fragments thereof, all as defined above, which are biotinylated or contain cysteine bridges. Biotinylated peptides can be obtained by any method known in the art, such as the one described in WO93/18054 to De Leys. Methods for obtaining peptides containing inter- and/or intramolecular cysteine bridges are extensively described in WO 96/13590 to Maertens & Stuyver.

The present invention also relates to any combination of peptides represented by SEQ ID 1 to 38 and functionally equivalent variants or fragments thereof as defined above. The terms "any

combination" refers to any possible mixture of above-described peptides or any possible linkage (covalently or otherwise) between above-described peptides. Examples of the latter peptide combinations are simple mixtures, homo- or hetero-branched peptides, combinations of biotinylated peptides presented on streptavidin, avidin or neutravidin, chemically cross-linked peptides with or without spacer, condensed peptides and recombinantly produced peptides.

The present invention relates also an antibody, more particularly a monoclonal antibody, characterized in that it specifically recognizes an HCV-related virus polypeptide as described above.

The present invention also relates to a method for diagnosing exposure to or infection by HCV-related viruses comprising contacting anti-HCV-related virus antibodies within a sample of body fluid with a peptide as described above or with a combination of peptides as described above, and, determining the binding of anti-HCV-related virus antibodies within a sample of body fluid with a peptide as described above or with a combination of peptides as described above.
As used herein, the term "a method for diagnosing" refers to any immunoassay known in the art such as assays which utilize biotin and avidin or streptavidin, ELISAs and immunoprecipitation and agglutination assays. A detailed description of these assays is given in WO 96/13590 to Maertens & Stuyver.

In this regard, the present invention also relates to an assay kit for detecting the presence of anti-HCV-related virus antibodies comprising a solid support, a peptide as described above or a functionally equivalent variant or fragment thereof, or combination of peptides as described above, and appropriate markers which allow to determine the complexes formed between anti-HCV-related virus antibodies within a sample of body fluid with a peptide as described above, or a functionally equivalent variant or fragment thereof, or combination of peptides as described above.

The term "a solid support" refers to any solid support known in the art.
Similarly, the term "appropriate markers" refers to any marker known in the art.
It should also be clear that the term "a method for diagnosing" encompasses screening, detection,

confirmation, monitoring and serotyping methods.

The present invention further pertains to a bioassay for identifying compounds which modulate the binding between a peptide and an anti-HCV-related virus antibody, comprising contacting anti-HCV-related virus antibodies with a peptide as described above, or a combination of peptides as described above, and determining the binding of anti-HCV-related virus antibodies with a peptide as described above, or a combination of peptides as described above, adding a modulator or a combination of modulators to the contacted anti-HCV-related virus antibodies with a peptide as described above, or a combination of peptides as described above, and finally determining the modulation of binding of anti-HCV-related virus antibodies with a peptide as described above, or a combination of peptides as described above.

In another embodiment the present invention features a bioassay for identifying compounds which modulate the binding between a peptide and an anti-HCV-related virus antibody, comprising determining the binding of anti-HCV-related virus antibodies with a peptide as described above, or a combination of peptides as described above, contacting a modulator with a peptide as described above, or a combination of peptides as described above, adding anti-HCV-related virus antibodies to the contacted modulator with a peptide as described above, or a combination of peptides as described above, determining the modulation of binding of anti-HCV-related virus antibodies with a peptide as described above, or a combination of peptides as described above.

The term "compound" as used herein refers to a composition, which has a molecular weight of less than about 25 KDa, preferably less than 10 KDa, and most preferably less than 5 KDa. Compounds can be nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules, or antibodies which may be generated by the host itself upon vaccination.

The term "binding" as used herein indicates that a peptide as described above is physically connected to, and interacts with antibodies. Binding of the peptide to the antibody can be demonstrated by any method or assay known in the art such as binding-, ELISA, and RIA-type of assays or competition assays (eg see Examples section and Current protocols in immunology).

The terms "modulation" or "modulate" as used herein refer to both upregulation (i.e., activation or stimulation (e.g., by agonizing or potentiating)) and downregulation (i.e. inhibition or suppression (e.g. by antagonizing, decreasing or inhibiting) of the binding between a peptide and an anti-HCV antibody.

The term "modulator" as used herein refer to the ability of a compound as described above to modulate as described above.

The term "peptidomimetics" as used herein refers to molecules which can be manufactured and which mimic those residues of peptides which modulate the interaction of the antibody with the peptide as described above. For instance, non-hydrolyzable peptide analogs of such residues can be generated using benzodiazepine (e.g., see Freidinger et al. in Peptides: Chemistry and Biology, G. R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), azepine (e.g., see Huffman et al. in Peptides: Chemistry and Biology, G. R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), PNA, substituted gamma lactam rings (Garvey et al. in Peptides: Chemistry and Biology, G. R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), ketomethylene pseudopeptides (Ewenson et al. (1986) J Med Chem 29:295: and Ewenson et al. in Peptides: Structure and Function (Proceedings of the 9th American Peptide Symposium) Pierce Chemical Co. Rockland, Ill., 1985), β-turn dipeptide cores (Nagai et al. (1985) Tetrahedron Lett
26:647; and Sato et al. (1986) J Chem Soc Perkin Trans 1:1231), and β-aminoalcohols (Gordon et al. (1985) Biochem Biophys Res Commun, 126:419; and Dann et al. (1986) Biochem Biophys Res Commun 134:71).

The present invention pertains to a modulator produced by a bioassay as described above.

The present invention pertains also to a modulator for the interaction between a peptide and an anti-HCV-related virus antibody, when said modulators are identified by a bioassay as described above.

The present invention features a composition comprising as an active substance a peptide as described above or a combination of peptides as described above.

The present invention features also a composition comprising as an active substance a modulator as described above or a combination of modulators as described above.

In another embodiment, the present invention relates to a composition comprising a plasmid vector comprising a nucleotide sequence encoding a peptide as described above, operably linked to transcription regulatory elements. Upon introduction in a human tissue said plasmid vector induces the expression in vivo, of the nucleotide sequence thereby producing the encoded protein. If said protein elicits an immunogenic response it is referred to as a DNA vaccine. It is readily apparent to those skilled in the art that variations or derivatives of the nucleotide sequence can be produced which alter the nucleotide sequence. The altered polynucleotide may have an altered nucleic sequence, yet still encodes a protein as described above, and which reacts with anti-HCV-related virus antibodies, and is considered a to be functional equivalent.

In a preferred embodiment, the present invention relates to a composition as described herein for use as to vaccinate humans against infection with HCV-related virus or any mutated strain thereof.

In another preferred embodiment, the present invention relates to a composition as described herein for use as to therapeutically treat humans against infection with HCV-related virus or any mutated strain thereof.

A composition of the present invention can be, as appropiate, any of the preparations described herein, including peptides, functionally equivalent variants or fragments thereof, a combination of peptides, or modulators (e.g. as identified in the bioassay provided herein). Specifically, the term "a composition" relates to an immunogenic composition capable of eliciting protection against HCV-related virus, in particular against HCV and/or HGV, whether partial or complete. The term "as an active substance" relates to the component of the vaccine composition which elicits protection against HCV-related viruses, in particular against HCV and/or HGV. An active substance (e.g. the peptides or the modulators of the present invention) can be used as such, in a biotinylated form (as explained in WO 93/18054) and/or complexed to *Neutralite Avidin* according to the manufacturer's instruction sheet (Molecular Probes Inc., Eugene, OR).

It should also be noted that "a composition" comprises, in addition to an active substance, a suitable excipient, diluent, carrier and/or adjuvant which, by themselves, do not induce the production of antibodies harmful to the individual receiving the composition nor do they elicit protection. Suitable carriers are typically large slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric aa's, aa copolymers and inactive virus particles. Such carriers are well known to those skilled in the art. Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: aluminium hydroxide, aluminium in combination with 3-0-deacylated monophosphoryl lipid A as described in WO 93/19780, aluminium phosphate as described in WO 93/24148, N-acetyl-muramyl-L-threonyl-D-isoglutamine as described in U.S. Patent N° 4,606,918, N-acetyl-normuramyl-L-alanyl-D-isoglutamine,N-acetylmuramyl-L-alanyl-D-isoglutamyl-L-alanine2(1'2'dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy) ethylamine and RIBI (ImmunoChem Research Inc., Hamilton, MT), which may contain one or all of the following elements: monophosphoryl lipid A (detoxified endotoxin), trehalose-6,6-dimycolate, and cell wall skeleton (MPL + TDM + CWS) in a 2% squalene/Tween 80 emulsion. Any of the three components MPL, TDM or CWS may also be used alone or combined 2 by 2. Additionally, adjuvants such as Stimulon (Cambridge Bioscience, Worcester, MA), MF 57 (Chiron) or SAF-1 (Syntex) may be used,as well as adjuvants such as combinations between QS21 and 3-de-O-acetylated monophosphoryl lipid A (WO94/00153), or MF-59 (Chiron), or poly[di(carboxylatophenoxy) phosphazene] based adjuvants (Virus Research Institute), or blockcopolymer based adjuvants such as Optivax (Vaxcel) or GammaInulin (Anutech), or Gerbu (Gerbu Biotechnik). Furthermore, Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) may be used for non-human applications and research purposes. "A composition" will further contain excipients and diluents, which are inherently non-toxic and non-therapeutic, such as water, saline, glycerol, ethanol, wetting or emulsifying agents, pH buffering substances, preservatives, and the like. Typically, a vaccine composition is prepared as an injectable, either as a liquid solution or suspension. Solid forms, suitable for solution on, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or encapsulated in liposomes for enhancing adjuvant effect. The polypeptides may also be incorporated into Immune Stimulating Complexes together with saponins, for example Quil A (ISCOMS). Compositions, which can be used as a vaccine, comprise an immunologically effective amount of the polypeptides of the present

invention and/or modulators, as well as any other of the above-mentioned components. "Immunologically effective amount" means that the administration of that amount to an individual, either in a single dosis or as part of a series, is effective for prevention or treatment. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated (e.g. nonhuman primate, primate, etc.), the capacity of the individual's immune system to mount an effective immune response, the degree of protection desired, the formulation of the vaccine, the treating's doctor assessment, the strain of the infecting HCV and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. Usually, the amount will vary from 0.01 to 1000 µg/dose, more particularly from 0.1 to 100 µg/dose.Compositions, which can be used as a vaccine are conventionally administered parenterally, typically by injection, for example, subcutaneously or intramuscularly.

In the case of DNA vaccines, particular useful methods for eliciting an immune response include the coating of gold particles with the plasmid vector encoding the desired peptide, and injecting them under high pressure into the epidermis and/or dermis, eg by means of a device called gene gun (eg as produced by Powderject Vaccines, Madison, WI, USA).

Additional formulations suitable for other methods of administration include oral formulations and suppositories. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents. It should be noted that a vaccine may also be useful for treatment of an individual, in which case it is used as a to "therapeutically treat humans".

As used herein, a "plasmid vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they have been linked.In general, but not limited to those, plasmid vectors are circular double stranded DNA loops which, in their vector form, are not bound to the chromosome. For expression purposes, promoters are required. For DNA vaccination, a very suitable promoter is the Major Immediate Early (MIE) of human cytomegalovirus.

As used herein, a "nucleotide sequence" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and single

(sense or antisense) and double-stranded polynucleotides.

As used herein, the term "transcription regulatory elements" refers to a nucleotide sequence which contains essential regulatory elements, ie such that upon introduction into a living vertebrate cell it is able to direct the cellular machinery to produce translation products encoded by the polynucleotide.

The term "operably linked" refers to a juxtaposition wherein the components are configured so as to perform their usual function. Thus, transcription regulatory elements operably linked to a nucleotide sequence are capable of effecting the expression of said nucleotide sequence. Those skilled in the art can appreciate that different transcriptional promoters, terminators, carrier vectors or specific gene sequences may be used succesfully.

Finally, the present invention provides a method to immunize humans against infection with HCV-related virus or any mutated strain thereof, comprising the use of a peptide as described above or a combination of peptides as described above.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and can not be construed as to restrict the invention in any way.

EXAMPLES

Example 1. Synthesis of multimer E1 and E2 peptides

We aimed at synthesizing peptides which would display epitopes, similar to the ones present on E1 and E2 peptides expressed in mammalian cells. Since such epitopes do not seem to be present in E1 and E2 proteins expressed in *E. coli*, the design of such peptides was not an easy task. We first aligned E1 and E2 primary amino acid sequences of different HCV genotypes and delineated variable and constant domains. It was reasoned that these domains, or a combination of two or more of these domains might represent conformational domains, ie form or constitute independent conformational units. If displayed as 3D structure, these conformational domains may also contain conformational epitopes. The latter domains may

therefore be able to adopt a native-like structure as is present in the envelope proteins when these envelope proteins are expressed in mammalian cells. In contrast, such structures are absent when the envelope proteins are expressed in prokaryotic cells, like *E.coli*.

The following domains were assigned:

V1, V2, V3, V4, V5, V6 = variable regions; C1, C2, C3, C4 = conserved domains; HR = hydrophobic region; SA = signal anchor sequence; HVRI, HVRII = hypervariable regions of E2.

Protein	Region	Amino acid position
E1	V1	192-203
	C1	204-217
	V2	218-223
	C2	224-229
	V3	230-242
	C3	243-247
	V4	248-257
	HR	258-293
	V5	294-303
	C4	304-329
	V6	330-342
	SA	343-383

Protein	Region	Amino acid position
E2	HVRI	384-411
	C1	412-470
	HVRII	471-482
	C2	483-521
	V3	522-548
	C3	549-569
	V4	570-580
	C4	581-704
	SA	705-746

Based on these domains of the BE11 subtype 1b isolate (SEQ ID 50 in PCT/EP 95/03031), we designed a series long peptides of 24 to 45 amino acids. For some extended domains of the envelope proteins more than one multimer peptide was synthesized in order to encompass the domain of interest. Table 1 gives an overview of the peptides with their respective amino acid positions; numbering starts from the first initiation codon of the HCV polyprotein. Peptides were synthesized using t-Boc technology as explained in detail in WO 93/18054.

Example 2. Reactivity of multimer peptides with E1 and E2 antibodies in patient sera

A series of 60 randomly chosen samples from patients with chronic active hepatitis C were tested for reactivity with the multimer peptides. These samples did not show any notable reactivity with 20-mer peptides except for some 20-mer peptides derived from the HVRI. For

comparison, reactivity with the hydrophylic ectodomain of E2, the recombinant E2h protein, was assayed (E2h extends from aa 384-708 and was cloned from SEQ ID NO 45, and expressed and purified as described in PCT/EP 95/03031). Peptides were coated onto streptavidin-coated plates (5µg/ml) and antibodies in serum samples were left to react and detected using the reagents and procedures as described in the package insert of the INNOTEST HCV Ab III kit (Innogenetics, Gent, Belgium). Table 2 shows the results of the ELISA tests, in which a cutoff of 150 mOD was used. In this series, 5 sera did not show reactivity with the E2h protein, only one of these reacted with the HVRI peptide. Five out of 60 sera (8%; e.g. sample 17758) only reacted with the E2h protein,

34 (57%) recognized HVRI, 24(40%) reacted with C1-a, 18 (30%) with C1-b, 21 (35%) with HVRII, 17 (28%) C2-a, 22 (37%) with C2-b, 18 (30%) with C3, 18 (30%) with C3', 17 (28%) with C3", 18 (30%) with V4, 22 (37%) with C-4, 21 (35%) with C4-a, 35 (58%) with C4-b, and 24 (40%) with C4-c. This experiment surprisingly learned that, while none of the samples recognized any of the 20-mer peptides, except for those derived from the HVRI, 50 out of 55 (91%) E2h reactive sera could be detected using the peptides of the present invention.

In a second series of 23 sera derived from chronic hepatitis C patients who were long-term responders to interferon-alpha treatment and 3 HCV infected chimpanzees, E1 and E2 antibodies were tested. Eighteen out of 23 samples (78%) reacted with recombinant E1s protein, expressed and purified from mammalian cells as described in PCT/EP 95/03031. Nine samples (39%) reacted with the C4V6 region, another 9 (39%) with the V1V2 region, and 3 with V2V3 (Table 4). For comparative purposes peptide V5, ie SQLFTISPRRHETVQD, is shown.

Different reactivities to E2 were observed (Table 4) as compared with the first series of samples. 21 samples (91%) reacted with E2h, with 13 (57%) reactive on HVRI, 9 (39%) with C1-a, 11 (48%) with C1-b, 1 with HVRII, C2-a, and C2-b each, 2 with C3, 3 with C4-a, 4 (17%) with C4-b, and 4 (17%) with C4-c. In this series of patients with a benign evolution of disease, the C1 region was more frequently recognized and fewer antibodies to the C4 region were detected as compared to the series of samples obtained from patients with chronic active hepatitis. These results indicate that peptides from the C1, C2, and C4 regions may be particularly useful in monitoring the course on HCV-related virus disease. More specifically, antibodies to the C1 region may better neutralize HCV as compared to anti-C4 antibodies. The C1 domain may therefore be functionally important, eg exhibit receptor-binding activity.

Neutralization of this region may therefore result in lesser activity of the disease and may lead to resolvement. The E2-C1 region may therefore be particularly useful in therapeutic interventions. It should also be noted that, once reactivity to a given domain is established, it can be further mapped to smaller peptides, e.g. reactivities of 1 chimpanzee serum to C3 could be mapped to smaller region of 25 amino acids (peptide C3").

Example 3. Monitoring of E1 and E2 antibodies in patients with response to interferon-alpha therapy

In Table 5, results of E2 antibody tests as described in example 2 are given for consecutive samples obtained from patients with response to interferon therapy. A decline in E2Ab, and to a larger extend E1Ab, has been described in PCT/EP 95/03031 in case of a long-term response to interferon treatment. Reactivities to several peptides of the present invention also show similar declining levels. Peculiar reactivities could sometimes be detected as exemplified in patient 2: upon the detection of reappearing virus, antibody responses to the (E1)V4V5 region and the (E2)HVRII region could be detected; these quickly disappeared simultaneously with viral clearance. (E1)V4V5 and (E2)HVRII may therefore be particularly useful peptides for disease monitoring, especially in treatment of disease. Other peptides such as (E2)C1 (example 2) and those shown in bold in Table 5 also seem to be useful for purposes such as monitoring. Table 2 also shows the presence of reactivity in patient 2 to a new peptide HVRI-C1, which overlaps the junction between HVRI and C1 (Table 2), in the absence of detectable reactivity to the HVRI or C1 peptides. Similarly, peptide C4-bc encompassing the region between C4-b and C4-c (Table 2), was tested in this series, and showed almost identical reactivities as compared to peptide C4-b. Therefore, it is possible that the C4-b epitope lies between aa 658 and 673, but surprisingly, the epitope does not seem to be presented in peptide SEQ ID 92 of PCT/EP 95/03031 (aa 655-674). The C4-c epitope is not present in C4-bc and therefore can be localized between aa 683 and 706.

Example 4: Application to other flaviviruses

To examine the applicability of the invention to envelope proteins of other HCV-related viruses, a peptide spanning the V1V2 region of the hepatitis G virus (GBV-C; Linnen et al.,

1996; Simons et al., 1996) E1 region was synthesized, see also SEQ ID NO 38 (Table 1):

NH2-THACRANGQYFLTNCCAPEDIGFCLEGGCLVALGGK-biotin .

So far, only reactivity to the complete HGV E2 protein seemed to be useful in the diagnosis of HGV. Peptide epitopes have not yet been described for GBV envelope proteins E1 or E2. Sixteen HGV RNA-positive sera were tested and 1 of these was reactive with the E1 peptide as shown in Table 6. Antibody reactivity to the recombinant HGV E2 protein (but not to HGV E2 peptides) is found in up to 15% of the European population, but cases with both HGV RNA and E2Ab are rare as they probably represent cases in which seroconversion and elimination of the virus is ongoing. Antibody reactivity to the HGV E1 protein has not yet been reported. Therefore, the HGV E1 peptide V1V2 is new and it may display higher reactivities in a series of HGV anti-E2 reactive sera. Using similar approaches as described in the present invention, HGV E2 peptides may also be synthesized. Multimer peptides from GBV-A or GBV-B can be synthesized in a similar approach as described for HCV and HGV.

Example 5: Reactivity of 20-mer E2 peptides compared to multimer E2 peptides.

E2 peptides listed in Table 1 were analyzed for their reactivity with 32 serum samples from patients with chronic active hepatitis C. In addition, a series of overlapping 20-mer peptides were synthesized with exactly the same HCV subtype 1b sequence as used for the longer peptides and as shown in Table 1. The ELISA test used was the same as described in Example 2. Figures 3 and 4 show the reactivities of the series of 20-mer and longer peptides, respectively. Peptides with a sum of >5 (HVR I, HVR I/C1, C1a, C1b, C4a, C4b, C4c, C4b-c) were considered to be very useful for the detection of antibodies directed against E2. A total of six of these peptides (peptides C4b-c and C1a were not included as these peptides are almost entirely represented by other peptides) were combined together with 20-mer peptide 1350 (Table 1), which occasionally reacted with some patient sera. The combination of these peptides was tested on a panel of 128 sera from chronic active HCV carriers. Hundred and twenty six of these sera tested positive on recombinant E2s protein. Of these 126 sera, 33 sera showed at least two times higher OD values with the peptide mixture as compared to the recombinant E2 protein, 64 sera showed a similar reactivity, 16 sera showed reactivities which were 2- to 4-fold higher with the recombinant protein than with the peptide mixture, and 13 sera only reacted with the recombinant protein.

In summary, almost 90% of the sera containing antibodies against recombinant E2 protein could be detected using the above peptide mixture. For 26% of the sera, detection was even better using the peptides of the invention, than using recombinant E2 protein. A sum of OD values of >5, ie exhibited by peptides HVR I, HVR I/C1, C1a, C1b, C4a, C4b, C4c, and C4b-c (Figure 4) is therefore considered a surprisingly high value for the serodiagnosis of antibodies directed against the E2 protein of HCV. From the experiment described above, it is also clear that a combination of recombinant E2 with the peptides of the invention is a particularly useful composition.

Given the variability of the E2 protein in different HCV genotypes, the addition of genotype-specific peptides to recombinant E2 proteins may be a desired way of improving sensitivity of E2 antibody assays. For example, a variant of peptide C1a based on a reported HCV type 2a sequence HC-J6 could be

LINTNGSWHINRTALNCNDSLHTGFLASLFYTHSF, and similar useful variants e.g. based on a genotype 3a sequence, could be synthesized and tested for reactivity. It should be noted that the HCV E2 protein may contain insertions or deletions in any given HCV genotype. For example, while subtype 1a and 1b sequences show contiguous sequences which can be aligned without having to insert gaps, HCV type 2a isolates encode E2 proteins which are 4 aa's longer as compared to type 1 sequences. For example, 2 additional amino acids are inserted in HCV type 2a and 2b sequences around hypervariable region II (HVR II). Therefore, a potentially useful variant of peptide HVRII, based on the HC-J6 prototype 2a sequence, would be

RSIEAFRVGWGALQYEDNVTNPEDMRPYCW, which is a 30-mer peptide while the subtype 1b sequence-based peptide depicted in Table 1 (SEQ ID 20) is only 28 aa's long. The two glutamates (symbol E) which are inserted in the subtype 2a sequence are shown underlined. Similar peptides can be easily constructed based on sequences and alignments previously published (e.g. Maertens and Stuyver, 1997).

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Table 1

PROTEIN	GENO TYPE	PEPTIDE	AMINO ACID SEQUENCE	POSITION	SEQ ID NUMBER
E1	1a	V1V2T1a	YQVRNSTGLYHVTNDCPNSSIVYEAADAILHTPGC	192-226	Seq ID 1
	1b	V1V2T1b	YEVRNVSGIYHVTNDCSNSSIVYEAADMIMHTPGC	192-226	Seq ID 2
	2c	V1V2T2c	VEVKNNSNSYMATNDCSNSSIIWQLEGAVLHTPGC	192-226	Seq ID 3
	2c	V1V2T2c'	VEVKNTSTSYMVTNDCSNSSIVWQLEGAVLHTPGC	192-226	Seq ID 4
	3a	V1V2T3a	LEWRNTSGLYVLTNDCSNSSIVYEADDVILHTPGC	192-226	Seq ID 5
	3a	V2T3a	LTNDCSNSSIVYEADDVILHTPGC	203-226	Seq ID 6
	4c/4k	V1V2T4a	INYRNVSGIYHVTNDCPNSSIVYEADHHILHLPGC	192-226	Seq ID 7
	5a	V1V2T5a	VPYRNASGIYHITNDCPNSSIVYEADNLILHAPGC	192-226	Seq ID 8
	6a	V1V2T6a	LTYGNSSGLYHLTNDCSNSSIVLEADAMILHLPGC	192-226	Seq ID 9
	1b	V2V3	IVYEAADMIMHTPGCVPCVRENNSSRCWV	212-240	Seq ID 10
	1b	V3V4	VRENNSSRCWVALTPTLAARNASVPTTTIRRHVD	230-263	Seq ID 11
	1b	PC-V3V4	PCVRENNSSRCWVALTPTLAARNASVPTTTIRRHVD	228-263	Seq ID 12
	1b	HR	HVDLLVGAAAFCSAMYVGDLCGSVFLVSQL	260-290	Seq ID 13
	1b	V5C4	SQLFTISPRRHETVQDCNCSIYPGHITGHRMAWDMMMNWS	288-327	Seq ID 14
	1b	C4V6	SIYPGHITGHRMAWDMMMNWSPTTALVVSQLLRI	307-340	Seq ID 15
	1b	SA	PQAVVDMVAGAHWGVLAGLAYYSMVGNWAKVLVVMLLFAGV	341-381	Seq ID 16
	1b	V4V5	VALTPTLAARNASVPTTTIRRHVDSQLFTISPRRHETVQD	240-303	Seq ID 37

Table 1 - cont'd 1

PROTEIN	GENO TYPE	PEPTIDE	AMINO ACID SEQUENCE	POSITION	SEQ ID NUMBER
E1(HGV)	ND	V1V2	THACRANGQYFLTNCCAPEDIGFCLEGGCLVALGGK	ND	Seq ID 38
E2	1b	HVR I	HTRVSGGAAASNTRGLVSLFSPGSAQKIQLVN	384-415	Seq ID 17
	1b	C1a	LVNTNGSWHINRTALNCNDSLQTGFFAALFYKHKF	413-447	Seq ID 18
	1b	C1b	NDSLQTGFFAALFYKHKFNSSGCPERLASCRSIDKFAQ	430-467	Seq ID 19
	1b	HVR II	RSIDKFAQGWGPLTYTEPNSSDQRPYCW	460-487	Seq ID 20
	1b	C2a	SDQRPYCWHYAPRPCGIVPASQVCGPVYCFTPSP	480-513	Seq ID 21
	1b	C2b	SQVCGPVYCFTPSPVVVGTTDRFGVPTYNWG	500-530	Seq ID 22
	1b	V3C3	GVPTYNWGANDSDVLILNNTRPPRGNWFGCTWMNGTGFTKTCGG	523-566	Seq ID 23
	1b	V3C3'	ANDSDVLILNNTRPPRGNWFGCTWMNGTGFTKTCGG	531-566	Seq ID 24
	1b	C3"	TRPPRGNWFGCTWMNGTGFTKTCGG	542-566	Seq ID 25
	1b	V4	TKTCGGPPCNIGGAGNNTLTCPTDCFRKHP	561-590	Seq ID 26
	1b	C4	TDCFRKHPEATYARCGSGPWLTPRCMVHYPYRLWHYPCTVNFTIF	583-627	Seq ID 27
	1b	C4'	ARCGSGPWLTPRCMVHYPYRLWHYPCTVNFTIF	595-627	Seq ID 28
	1b	C4"	LTPRCMVHYPYRLWHYPCTVNFTIF	603-627	Seq ID 29
	1b	C4a	TVNFTIFKVRMYVGGVEHRFEAACNWTR	621-648	Seq ID 30
	1b	C4b	EAACNWTRGERCDLEDRDRSELSPLLLSTTEWQ	641-673	Seq ID 31
	1b	C4c	QWQILPCSFTTLPALSTGLIHLHQNIVDVQYLYGVG	671-706	Seq ID 32

Table 1 - cont'd 2

PROTEIN	GENO TYPE	PEPTIDE	AMINO ACID SEQUENCE	POSITION	SEQ ID NUMBER
E2	1b	SA	GVGSAVVSLVIKWEYVLLLFLLLADARICACLWMMLLIAQAE	704-745	Seq ID 33
	1b	HVR I/C1	NTRGLVSLFSPGSAQKIQLVNTNGSWHINRTALN	395-428	Seq ID 34
	1b	C4b-c	DRSELSPLLLSTTEWQILPCSFTTLPALSTG	658-688	Seq ID 35
	1b	1350	VGTTDRFGVPTYNWGANDSD	516-535	Seq ID 36

Table 2

Sample #	HVR I	C1-a	C1-b	HVR II	C2-a	C2-b	E2-13 B	C3	C3'	C3"	V4	C4	C4-a	C4-b	C4-c	SA	R c E2
17758	69	48	47	52	49	48	47	49	38	44	43	52	44	55	48	46	1355
17763	88	54	44	49	52	48	51	51	46	45	48	49	45	133	104	50	361
17764	100	148	138	134	128	136	141	136	136	65	130	145	144	242	128	127	371
17766	91	97	145	96	80	87	90	90	95	47	75	89	163	139	99	86	173
17771	307	79	54	65	51	50	65	68	50	45	60	65	59	96	132	58	393
17775	49	50	46	39	50	271	43	51	48	45	50	55	52	54	47	50	228
17777	60	133	105	130	129	123	118	118	130	95	119	133	129	357	177	113	850
17779	373	328	285	330	284	343	281	323	316	283	297	318	343	341	309	282	720
17785	81	80	73	71	76	66	81	70	74	70	69	79	79	87	119	73	146
17786	341	863	693	152	164	179	148	139	146	136	137	158	160	163	148	157	720
17788	111	553	120	137	69	121	121	119	111	110	103	140	132	131	48	47	934
17789	1316	49	47	46	49	45	53	51	48	43	42	50	49	52	48	48	1178
17790	234	233	182	223	130	224	185	185	186	184	179	216	218	1347	853	207	1534
17791	269	194	177	192	123	203	172	192	157	184	184	200	195	211	187	190	287
17797	260	264	248	257	240	281	249	237	246	221	223	283	261	272	231	243	1357
17798	52	53	50	47	52	54	50	53	49	51	50	51	50	1036	51	51	1161
17799	225	89	81	86	85	100	76	85	87	82	84	86	92	115	86	76	362
17802	42	51	44	47	50	133	48	52	51	48	51	56	76	773	157	56	882
17807	49	133	60	59	66	62	62	59	57	56	57	63	65	62	57	52	605
17808	89	121	117	109	106	1051	118	875	133	116	123	126	393	228	109	126	1354

Table 2 - cont'd 1

17810	327	220	199	222	195	200	221	182	197	182	196	209	266	222	195	199	422
17818	224	134	115	126	118	115	128	108	109	98	111	113	112	117	109	108	230
17821	671	243	214	282	238	232	228	217	234	197	216	222	218	557	810	205	1046
17825	397	320	264	284	282	286	289	277	276	274	276	306	273	391	399	277	514
17826	92	109	111	99	114	126	113	98	104	84	105	121	122	126	145	113	695
17827	45	47	46	47	48	49	48	49	49	47	49	50	50	261	113	47	320
17832	151	65	55	70	78	63	77	72	68	59	64	70	62	54	57	49	288
17838	212	167	166	164	156	165	164	146	160	154	150	165	165	161	272	157	305
17839	48	94	117	61	61	51	58	51	46	52	58	55	87	60	95	66	182
17840	318	323	347	317	329	338	320	305	326	302	312	343	355	322	318	337	417
17842	161	174	185	176	168	163	159	157	163	156	150	168	151	154	138	153	195
17844	122	94	90	88	98	78	92	88	84	77	85	94	61	214	51	73	166
17849	1469	68	75	49	54	629	52	53	46	46	51	54	119	1102	55	47	1393
17870	125	236	148	114	128	133	135	116	132	109	135	151	118	293	120	45	197
17879	209	195	201	222	195	215	225	191	194	181	218	209	209	255	253	199	325
17983	438	54	50	48	52	46	50	54	46	46	51	52	46	55	53	48	216
17999	276	201	200	202	190	187	191	169	176	150	190	205	186	321	535	198	697
8242	162	114	114	127	140	114	120	117	103	120	117	107	112	161	152	128	340
8243	188	191	171	175	204	172	189	174	186	174	176	205	200	206	177	178	225
8247	248	169	137	127	120	110	122	96	111	104	114	128	104	130	150	118	215
8250	129	161	127	150	164	144	154	125	134	122	142	151	125	146	137	140	165
8317	112	131	115	123	113	111	144	95	103	95	108	118	108	158	126	111	198
8320	463	433	337	473	435	445	363	345	503	384	362	369	405	446	432	378	474
8329	119	126	123	160	143	145	142	117	135	121	122	126	131	152	148	132	163
8330	198	271	210	210	207	196	216	178	194	206	209	215	186	356	45	51	536

Table 2 - cont'd 2

8332	154	141	128	141	132	116	129	110	123	112	135	140	123	147	312	144	290
8333	57	67	50	51	52	52	50	54	50	50	50	56	48	480	65	52	1108
8334	283	66	64	80	68	69	84	79	65	52	67	74	72	180	191	90	348
8337	162	105	99	108	103	92	104	86	93	80	101	107	108	124	118	110	142
8339	50	49	52	62	54	46	54	51	47	41	51	55	53	413	49	50	247
8344	59	52	50	51	58	48	54	52	47	48	55	53	58	63	63	60	59
8351	163	114	105	111	101	91	98	97	92	78	110	111	115	141	179	112	154
8362	211	54	50	47	55	119	53	53	44	45	51	54	59	60	58	55	165
8364	110	308	106	112	112	107	98	102	108	92	116	152	133	208	169	132	671
8365	69	84	94	67	77	74	55	73	70	69	70	79	73	69	88	66	86
8367	218	189	171	201	204	174	191	156	158	140	183	186	294	197	186	171	303
8374	575	113	95	114	110	93	100	92	106	88	103	125	118	112	111	106	143
8377	364	232	229	225	211	202	233	189	207	170	209	205	230	234	218	221	293
8382	314	211	187	196	207	173	208	181	158	150	181	187	201	223	189	211	265
8383	51	100	102	55	58	48	57	53	53	50	52	57	66	94	63	56	285
V1200	52	55	52	56	55	53	50	54	50	52	51	50	50	52	53	54	50
V1201	118	147	138	136	224	144	123	137	140	111	135	154	166	171	137	155	162
V1202	274	308	284	170	290	286	282	248	277	229	271	306	287	330	268	295	329
V1204	130	134	135	127	141	128	79	113	119	106	131	144	145	144	130	144	159

Table 3

		E1 antigens						
Sample#	No peptide	V1V2	V2V3	V3V4	HR/SA	V5	C4V6	rec E1s
No sample	0.011	0.007	0.011	0.014	0.009	0.007	0.009	0.056
30108	0.03	0.035	0.04	0.034	0.032	0.03	0.234	0.378
30109	0.032	0.033	0.035	0.028	0.024	0.026	0.227	0.368
30110	0.021	0.545	0.02	0.019	0.016	0.017	0.047	0.669
30111	0.017	0.614	0.019	0.018	0.017	0.015	0.064	0.796
30112	0.037	0.069	0.035	0.034	0.031	0.031	0.048	0.187
30113	0.042	0.083	0.136	0.039	0.034	0.035	0.063	0.226
30114	0.042	0.099	0.036	0.035	0.035	0.037	0.058	0.267
30115	0.021	0.114	0.023	0.021	0.02	0.02	0.189	0.339
30116	0.019	0.442	0.025	0.022	0.022	0.018	0.056	0.645
30117	0.027	0.062	0.047	0.043	0.041	0.038	0.066	0.164
30118	0.122	0.216	0.126	0.12	0.11	0.125	0.696	0.923
30119	0.023	0.028	0.031	0.028	0.023	0.024	0.23	0.426
30120	0.025	0.024	0.027	0.025	0.039	0.027	0.03	0.024
30121	0.03	0.033	0.033	0.029	0.052	0.034	0.037	0.032
30122	0.029	0.031	0.056	0.03	0.052	0.033	0.035	0.03
30123	0.085	0.081	0.076	0.075	0.087	0.071	0.094	0.137
30124	0.022	0.084	0.022	0.022	0.023	0.022	0.193	0.391
30125	0.095	0.128	0.091	0.089	0.172	0.159	0.47	0.708
17805	0.038	0.051	0.039	0.033	0.09	0.154	0.738	1.169
13059	0.011	0.011	0.012	0.012	0.014	0.012	0.229	0.681
Chimp1	0.095	0.38	0.276	0.126	0.098	0.095	0.099	0.805
Chimp2	0.026	0.234	0.143	0.035	0.036	0.038	0.354	0.822
Chimp3	0.018	0.017	0.02	0.022	0.023	0.019	0.141	0.353

Table 4.

Sample	E2 antigens peptide	HVR I	C1-a	C1-b	HVR II	C2-a	C2-b	C3	C3'	C3"	V4	C4	C4-a	C4-b	C4-c	recE2h
No sample	0.006	0.009	0.011	0.015	0.007	0.006	0.01	0.01	0.01	0.01	0.01	0.01	0.007	0.007	0.009	0.032
30108	0.036	0.747	0.848	0.969	0.032	0.033	0.03	0.04	0.02	0.02	0.03	0.03	0.041	0.026	0.031	0.988
30109	0.027	0.849	0.93	1.053	0.027	0.032	0.03	0.03	0.02	0.02	0.03	0.02	0.038	0.023	0.026	1.079
30110	0.018	0.026	0.021	0.044	0.019	0.024	0.02	0.02	0.02	0.02	0.02	0.03	0.023	0.026	0.056	0.11
30111	0.017	0.02	0.021	0.088	0.018	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.022	0.028	0.07	0.137
30112	0.037	0.092	0.052	0.177	0.044	0.048	0.04	0.04	0.04	0.04	0.04	0.05	0.043	0.562	0.053	0.947
30113	0.045	0.104	0.054	0.276	0.051	0.047	0.05	0.03	0.04	0.04	0.04	0.05	0.054	0.633	0.07	1.003
30114	0.045	0.112	0.075	0.726	0.046	0.041	0.05	0.05	0.03	0.04	0.04	0.06	0.054	0.646	0.067	1.065
30115	0.022	0.982	0.034	0.064	0.025	0.025	0.02	0.03	0.02	0.03	0.03	0.02	0.03	0.097	0.031	0.413
30116	0.015	0.023	0.02	0.04	0.017	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.023	0.022	0.046	0.084
30117	0.04	0.087	0.048	0.119	0.037	0.044	0.05	0.05	0.03	0.04	0.04	0.04	0.041	0.547	0.049	0.935
30118	0.112	0.213	0.122	0.119	0.119	0.121	0.12	0.12	0.11	0.05	0.11	0.1	0.117	0.105	0.2	0.289
30119	0.03	0.954	1.012	1.128	0.026	0.029	0.03	0.03	0.02	0.02	0.03	0.03	0.035	0.026	0.03	1.123
30120	0.031	0.427	0.208	0.208	0.03	0.033	0.03	0.04	0.03	0.03	0.03	0.03	0.033	0.032	0.032	0.577
30121	0.033	0.734	0.463	0.398	0.037	0.042	0.04	0.05	0.04	0.03	0.04	0.03	0.04	0.034	0.037	0.963
30122	0.03	0.661	0.413	0.365	0.043	0.034	0.03	0.04	0.04	0.03	0.03	0.03	0.038	0.03	0.034	0.907
30123	0.079	0.11	0.576	0.789	0.09	0.108	0.09	0.08	0.08	0.06	0.07	0.06	0.091	0.078	0.077	0.916
30124	0.02	0.939	0.041	0.065	0.028	0.237	0.04	0.04	0.02	0.03	0.02	0.02	0.038	0.108	0.049	0.4
30125	0.096	0.133	0.103	0.096	0.097	0.115	0.15	0.14	0.09	0.09	0.09	0.1	0.1	0.092	0.183	0.227
17805	0.042	0.255	0.074	0.078	0.071	0.045	0.06	0.06	0.05	0.04	0.06	0.04	0.163	0.043	0.831	0.881
13059	0.013	0.47	0.02	0.019	0.018	0.022	0.02	0.03	0.02	0.01	0.01	0.02	0.36	0.052	0.904	0.944
Chimp1	0.102	0.103	0.116	0.118	0.23	0.109	0.12	0.19	0.17	0.19	0.1	0.1	0.087	0.098	0.095	0.581
Chimp2	0.028	0.181	0.267	0.261	0.056	0.032	0.03	0.04	0.04	0.04	0.04	0.04	0.188	0.035	0.033	1.008
Chimp3	0.058	0.035	0.162	0.086	0.026	0.062	0.02	0.03	0.04	0.02	0.03	0.03	0.023	0.02	0.026	1.327

Tabl 5

Sample	HCV	*E1 peptides*								
Patient 1	PCR	Genotype	V1V2	V2V3	V3V4	V4V5	HR/SA	V5C4	C4V6	E1s
14/8/90	pos	3a	0.014	0.03	0.06	0.034	0.037	0.048	0.045	0.051
01/06/91			0.03	0.032	0.064	0.041	0.041	0.051	0.048	0.045
20/9/91	neg		0.06	0.064	0.064	0.037	0.039	0.05	0.398	0.045
13/3/92			0.034	0.041	0.037	0.034	0.037	0.046	0.044	0.04
04/09/92	neg		0.037	0.041	0.039	0.037	0.037	0.052	0.048	0.043
24/9/93			0.048	0.051	0.05	0.046	0.052	0.048	0.047	0.042
20/10/94	neg		0.045	0.048	0.398	0.044	0.048	0.047	0.045	0.041
23/10/95			0.051	0.045	0.045	0.04	0.043	0.042	0.041	0.051
10/12/96	pos?		0.037	0.041	0.033	0.034	0.035	0.039	0.038	0.045
Patient 2										
15/2/90			0.106	0.103	0.104	0.108	0.104	0.949	0.872	1.03
03/05/90	pos	1a	0.103	0.109	0.106	0.104	0.108	0.828	0.859	1.04
04/12/90			0.096	0.103	0.105	0.103	0.095	0.737	0.848	1.218
23/9/91			0.063	0.078	0.078	0.067	0.072	0.318	0.354	0.66
14/4/92			0.099	0.106	0.099	0.1	0.096	0.219	0.255	0.491
18/12/92			0.104	0.106	0.102	0.105	0.101	0.222	0.249	0.448
26/3/93			0.089	0.095	0.09	0.085	0.082	0.168	0.194	0.357
30/9/93	neg		0.092	0.081	0.089	0.09	0.088	0.17	0.18	0.35
17/6/94	pos	1a	0.084	0.09	0.096	0.599	0.095	0.154	0.166	0.32
18/12/95			0.072	0.077	0.077	0.077	0.081	0.111	0.121	0.206
23/12/96	neg		0.065	0.078	0.074	0.073	0.078	0.106	0.108	0.199
Patient 3										
15/04/93			0.005	0.006	0.005	0.004	0.006	0.005	0.006	0.007
06/09/94	pos	3a	0.007	0.008	0.007	0.008	0.007	0.006	0.006	0.009

Table 5 - cont'd 1

30/10/95	neg			0.007	0.01	0.009	0.009	0.009	0.008	0.007	0.011	
18/11/96	pos?	1b	0.012	0.012	0.012	0.011	0.01	0.009	0.009	0.012		
Patient 4												
12/04/91	pos	1a	0.006	0.007	0.006	0.006	0.007	0.006	0.006	0.01		
23/09/91	neg		0.01	0.01	0.008	0.009	0.009	0.006	0.008	0.013		
27/07/92	neg		0.007	0.009	0.007	0.008	0.007	0.006	0.007	0.01		
11/06/93	neg		0.009	0.011	0.009	0.01	0.009	0.007	0.006	0.011		
29/11/96	pos	1a	0.007	0.01	0.008	0.007	0.007	0.005	0.006	0.008		
Patient 5												
18/09/92	pos		0.017	0.01	0.008	0.007	0.008	0.178	0.196	0.537		
17/12/93	neg		0.012	0.014	0.011	0.01	0.011	0.039	0.04	0.231		
15/11/96	neg		0.012	0.014	0.012	0.01	0.01	0.026	0.017	0.116		
Patient 6												
10/05/90	pos		0.311	0.006	0.007	0.005	0.006	0.004	0.01	0.544		
11/10/91	neg		0.284	0.007	0.007	0.006	0.007	0.006	0.013	0.605		
Patient 7												
10/10/91	pos	1b	0.009	0.01	0.009	0.008	0.008	0.008	0.01	0.043		
18/12/92	neg		0.01	0.011	0.011	0.009	0.009	0.008	0.011	0.043		
28/06/93	neg		0.006	0.006	0.007	0.006	0.007	0.005	0.008	0.021		
10/03/97	pos	1b	0.008	0.008	0.007	0.008	0.007	0.006	0.008	0.012		
Patient 8												
19/08/91	neg		0.008	0.009	0.008	0.008	0.008	0.006	0.008	0.009		
17/07/95	pos	1b	0.01	0.009	0.009	0.009	0.006	0.007	0.007	0.018		
09/10/95	pos	1b	0.007	0.007	0.008	0.005	0.006	0.007	0.007	0.009		
15/12/95	neg		0.008	0.009	0.008	0.009	0.008	0.007	0.007	0.011		
04/03/96	neg		0.009	0.011	0.01	0.011	0.009	0.008	0.007	0.01		

Table 5 – cont'd 2

02/09/96	neg		0.01	0.011	0.011	0.01	0.01	0.008	0.008	0.013
Patient 9										
26/08/91	pos	1b/2ac	0.044	0.015	0.022	0.023	0.028	0.031	0.034	0.115
21/12/93	neg		0.033	0.017	0.021	0.027	0.022	0.025	0.023	0.048
20/12/94	pos	1b	0.023	0.016	0.015	0.028	0.019	0.028	0.034	0.077
21/12/95	pos	1b	0.019	0.029	0.024	0.027	0.027	0.031	0.034	0.048
Patient 10										
27/04/92	pos	1b	0.128	0.024	0.02	0.023	0.026	0.118	0.449	0.68
01/06/93	neg		0.107	0.03	0.029	0.027	0.026	0.098	0.385	0.667
Patient 11										
09/11/90	neg		0.018	0.019	0.012	0.013	0.015	0.087	0.141	0.591
12/07/91	pos	1b	0.023	0.023	0.016	0.02	0.018	0.073	0.1	0.466
28/05/93	pos		0.008	0.009	0.009	0.005	0.008	0.123	0.173	0.495
20/01/95	neg		0.011	0.009	0.008	0.007	0.007	0.026	0.047	0.187
08/01/96	neg		0.012	0.013	0.01	0.009	0.009	0.025	0.031	0.21
07/02/97	neg		0.019	0.019	0.014	0.014	0.013	0.027	0.051	0.203
Patient 12										
11/05/92	pos	1b	0.017	0.013	0.011	0.014	0.015	0.227	0.173	0.425
26/02/93	neg		0.022	0.014	0.013	0.013	0.014	0.178	0.264	0.417
12/08/93	pos	1b	0.016	0.016	0.016	0.014	0.015	0.29	0.387	0.63
Patient 13										
07/01/91	pos	1b	0.027	0.017	0.021	0.026	0.026	0.04	0.074	0.062
19/08/91	neg		0.018	0.018	0.015	0.013	0.012	0.021	0.009	0.043
21/08/92	pos		0.015	0.012	0.015	0.014	0.017	0.015	0.021	0.023
06/08/93	neg		0.019	0.018	0.016	0.021	0.016	0.01	0.011	0.02
06/03/95	pos	1b	0.027	0.026	0.018	0.015	0.018	0.02	0.023	0.028

Table 5 - cont'd 3

12/04/96	neg		0.03	0.017	0.018	0.036	0.021	0.027	0.027	0.022
Patient 14										
22/11/94	pos	1b	0.016	0.011	0.013	0.013	0.026	0.318	0.437	0.461
11/10/95	pos		0.024	0.014	0.014	0.018	0.019	0.039	0.061	0.059
15/02/96	neg		0.032	0.022	0.021	0.023	0.016	0.031	0.041	0.102
Patient 15										
04/12/90	pos	1b	0.003	0.005	0.005	0.004	0.005	0.005	0.005	0.019
29/11/90	neg		0.005	0.005	0.005	0.006	0.005	0.008	0.006	0.011
09/10/92	pos	1b	0.006	0.008	0.007	0.007	0.007	0.006	0.005	0.012
25/03/96	neg		0.006	0.008	0.007	0.006	0.006	0.004	0.007	0.012
Patient 16										
16/12/91	pos	3a	0.003	0.004	0.006	0.004	0.004	0.08	0.102	0.435
04/10/93	neg		0.006	0.007	0.007	0.006	0.008	0.028	0.033	0.253
12/09/94	neg		0.004	0.008	0.006	0.005	0.005	0.034	0.038	0.197
09/09/96	neg		0.004	0.008	0.007	0.006	0.005	0.008	0.013	0.08
Patient 17										
24/04/97	pos	1b	0.076	0.006	0.008	0.004	0.009	0.203	0.327	1.196
Patient 18										
08/01/97	neg		0.006	0.007	0.007	0.007	0.006	0.006	0.008	0.009
Blank			0.006	0.009	0.009	0.006	0.006	0.007	0.006	0.009

Table 6.

Sample#	Blank	E1 V1V2
20188	68	74
20189	77	73
20251	170	150
20252	490	1319
20253	92	70
20254	50	55
20255	81	88
20256	56	62
20266	119	134
20271	77	78
20272	61	69
21010	129	135
21011	159	161
21012	120	93
21286	108	105